Environmental enrichment induces optimistic cognitive bias in rats

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People’s affective or emotional state can alter their cognitive processing, biasing interpretation of ambiguous stimuli. Those in a more positive state interpret such stimuli in a more optimistic manner than those in a negative state. Recently, this research has extended to animals, and has shown that manipulations associated with negative affect cause animals to interpret ambiguous stimuli more pessimistically. We investigated whether exposure to environmental enrichment engenders optimistic responses to ambiguous stimuli. Rats, Rattus norvegicus, were trained on a novel conditional discrimination task whereby they learned the correct response necessary to obtain a food reward given the stimulus present during approach (rough or smooth sandpaper). One stimulus was associated with a higher value reward than the other. Once the rats were trained, cognitive bias was probed by exploring their responses to an ambiguous stimulus (intermediate grade of sandpaper); a rat was defined as optimistic if it chose the response appropriate to the stimulus associated with the better reward. Animals transferred from unenriched to enriched cages showed more optimistic responses following the change. A control group maintained in unenriched cages showed pessimistic responses throughout. These results demonstrate for the first time that environmental enrichment can induce an optimistic cognitive bias in rats previously housed in standard caging, possibly indicative of a more positive affective state. These results add support to the suggestion that measuring cognitive biases can give an insight into animal emotional states; this has implications for animal welfare and preclinical testing of potential therapeutics for mood disorders.

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A number of researchers have investigated whether environmental manipulations chosen to induce negative affect also produce pessimistic cognitive biases in animals’ responses to ambiguous stimuli (reviewed in Mendel et al. 2009). In the first of the studies, Harding et al. (2004) trained rats, Rattus norvegicus, to press a lever to obtain a food reward in response to one ‘training’ tone (the positive stimulus) and to refrain from pressing in response to a different ‘training’ tone (the negative stimulus) to avoid a burst of aversive white noise. After the rats had learned this discrimination, they were exposed to probe tones that were intermediate between the two trained tones. Those animals housed in unstable conditions (hypothesized to promote mildly depressive effects) showed longer response latencies and tended to respond less often to the ‘positive event’ tone and probe tones close to it. The authors interpreted these findings to suggest that unstable housing induces negative (or ‘pessimistic’) cognitive bias in rats. Following this pioneering study, a number of experimenters have further investigated how environmental manipulation affects judgement biases in animals. For example, removal of environmental enrichment or exposure to anxiety-inducing conditions increases ‘pessimistic’ judgements in both rats (Burman et al. 2008a, b, 2009) and European starlings, Sturnus vulgaris (Bateson & Matheson 2007; Matheson et al. 2008). Furthermore, both congenitally helpless rats (a genetic model of animal depression) and starlings with locomotor stereotypies (which are often interpreted as indicative of inadequate housing) show more pessimistic judgement biases than normal animals (Enkel et al. 2009; Brilot et al. 2010). Thus there is mounting evidence that cognitive bias could be a useful tool for assessing both state and trait negative affect in animals.

Despite evidence from humans that positive affect is associated with greater optimism (e.g. Nygren et al. 1996; Waters 2008), animal studies have so far failed to demonstrate clear optimistic judgement biases in response to manipulations designed to induce positive affect (Bateson & Matheson 2007). The aim of this study was therefore to explore whether cognitive bias can be used to measure positive affect in rats experiencing a sudden improvement in environmental conditions for the first time in their lives.

In this study we developed a novel cognitive bias test for rodents logically identical to two of the tasks previously used with starlings (Matheson et al. 2008; Brilot et al. 2010). Unlike the original Harding et al. (2004) go/no-go task described above, our rats were required to respond to both the positive and the negative cues by making an active choice response. We have argued that this design reduces the likelihood of confounds caused by changes in activity or motivation to feed inherent in a go/no-go task (Matheson et al. 2008; see also Enkel et al. 2009 for similar arguments). In the rat version of our task the positive stimulus (either fine- or coarse-grade sandpaper) was associated with a high-value reward (chocolate) and the negative stimulus (the opposite grade of sandpaper to the positive stimulus) with a lower-value reward (cereal). Rats will forage for both of these rewards, but are assumed to have a strong preference for chocolate over cereal. We chose not to use a punishment (white noise or electric shock) as in previous rat studies for two reasons: first, it has been hypothesized that tasks using positive and neutral reinforcers might be best at detecting positive affect or ‘happiness’ (Mendel et al. 2009); and second, we were concerned that repeated experience of punishers during daily cognitive bias testing might itself affect the state of the animals adversely. As enrichment has been used in previous animal tests of cognitive bias, and is widely thought to improve animal welfare (Garner 2005; Swaisgood & Shepherdson 2005), we tested the responses of rats to ambiguous probe cues before and after the addition of environmental enrichment, with the hypothesis that provision of enrichment would induce optimistic shift in cognitive bias. Rats were chosen for the study as they are among the most commonly used animals in biological research. Therefore, the successful development of the cognitive bias protocol in this species has profound implications both for the assessment of welfare and as a potential new research tool in the preclinical testing of mood-altering therapeutics.

**METHODS**

Subjects and Housing

We used 12 male Sprague Dawley rats (bred from an in-house colony and raised by their own mothers at the University of Edinburgh), approximately 6 months old at the start of testing. Post-weaning but before experiments began (i.e. weaning to 6 months), rats were housed in two groups of six in standard cages (61 x 43.5 cm and 21.5 cm high, Techniplast) lined with wood shavings (Lillico) but containing no environmental enrichment on a 12:12 h light:dark cycle and fed standard rat chow (RM1, Special Diet Services, Lillico, Surrey, U.K.) and water ad libitum. Temperature and humidity were maintained between 19 and 21 °C and 45 and 60%, respectively. Rats were identified using rings of permanent marker around the tail. Rats were killed using a schedule one method (cervical dislocation) at the end of the experiment.

Apparatus

In a room separate to the housing area we set up a simple maze consisting of a clear Perspex start box (61 x 43.5 cm and 21.5 cm high) connected to a clear Perspex goal box (61 x 43.5 cm and 21.5 cm high) via a large piece of white Perspex drainpipe (diameter 10 cm, length 80 cm). The goal box contained two foraging bowls (diameter 9 cm, height 5 cm) one black, one white, and the entire maze was set on a bench side (1 m high) under regular room lighting (Fig. 1).

Habitation

To habituate rats to the food rewards and maze apparatus, we handled each rat for 10 min daily and fed it food items to be used as rewards in the task (white chocolate drops and Honey Nut Cheerios) for 5 days (phase A). For the next 5 days, we also placed rats into the maze apparatus for 5 min per day (phase B). During this phase, the foraging bowls were filled with scented sand. One foraging bowl was filled with coriander-scented sand (1% by weight coriander), and the other with cinnamon-scented sand (1% by weight cinnamon). For each rat, each reward was specifically paired with a particular bowl colour and scent, and a particular bowl colour was either on the left or the right of the goal box, and this arrangement remained consistent for each individual throughout the experiment (e.g. chocolate reward always in black cinnamon bowl on left, Cheeroi reward always in white coriander bowl on right), but was randomized between individuals. These pairings were counterbalanced between treatment groups (enriched versus unenriched). The inside of the tunnel linking the start and goal boxes was completely lined with Silicon Carbide Waterproof sandpaper (3M, U.K.; P600 grade; this was different to the sandpaper used in later phases) to habituate rats to the presence of sandpaper in the tunnel. This complete lining facilitated maximal contact between the rat (feet and whiskers) and the surface of the sandpaper.

Training

During phase C, each rat underwent four training trials per day in the maze apparatus for a period of 5 days, two between 0900
hours and midday (am trials) and two between 1300 and 1700 hours (pm trials). During a trial, a reward (half a white chocolate drop or Honey Nut Cheerio) was placed on to the surface of the sand in the corresponding bowl. White chocolate drops were used as ‘high-value’ rewards and Honey Nut Cheerios as ‘low-value’ rewards. It was assumed that rats would value and be motivated to forage for both chocolate and Cheerios, as they are both sweet and it is well known that many mammals, including rats, have a strong preference for sweet foods (Vigues et al. 2009). Chocolate drops were assumed to be valued more highly than Cheerios for a number of reasons. First, they have greater calorific value and sugar content (around 3 kcal and 0.34 g sugar per half chocolate drop versus 0.4 kcal and 0.07 g sugar per half Cheerio). Second, we observed during habituation that rats ate the chocolate faster than the Cheerios. Furthermore, during all rewarded phases, rats exited the tunnel and located the reward faster for the chocolate compared with the Cheerio reward (see Results). Previous studies have shown that shorter choice latencies are correlated with preference during foraging (Bateson & Kacelnik 1995), and pilot data from one of our laboratories shows that rats prefer chocolate over Cheerios when given a straight choice (M. Bateson & M. Leach, personal observation). During a trial, rats were placed individually into the start box. The experimenter then started a timer and recorded the time taken for the rat to leave the start box and exit the tunnel, to choose a bowl (and which bowl was chosen: bowl with or without reward, correct and incorrect bowls, respectively), and to choose the reward (correct) bowl (if not chosen first). A choice was determined when a rat began digging in a particular bowl. Each rat received two trials for a chocolate drop, and two for a Cheerio per day. The order of these trials was determined independently by randomization, and was altered daily. Waterproof sandpaper of different grades (P60 coarse and P1200 fine; Faithfull Tools, Dartford, Kent, U.K.) was placed inside the tunnel and associated with the rewards. Half of the rats had coarse sandpaper (P60) during the chocolate trials and fine sandpaper (P1200) during the Cheerio trials, and vice versa for the other half of the individuals. This was counterbalanced between groups. This enabled rats to associate a particular grade of sandpaper with a particular reward, and so eventually choose the correct bowl first time upon entering the goal box (e.g. for one individual, coarse sandpaper was associated with chocolate located in a white cinnamon bowl on the right). To avoid possible effects of odour cues, sandpaper was changed between rats, and the apparatus cleaned with a small amount of 70% ethanol.

After completion of phase C, rats entered phase D. During phase D (5 days), the same protocol was followed as in phase C, except the depth at which the rewards were buried in the sand progressively increased, until they were at the bottom of the foraging bowl by day 5. Next rats entered phase E; again the same basic protocol was followed as in phase C, but here rewards were always buried at the bottom of the sand and one out of the four trials carried out each day (chosen at random) was not rewarded. Correct responses (a correct response was defined as the rat choosing the usually rewarded bowl) on these unrewarded trials would indicate that rats were not simply using cues from the reward itself (e.g. olfactory cues) to guide decisions. Rats were removed from the maze after their initial choice. Performance (i.e. correct versus incorrect responding) was not affected by the presence or absence of reward (see Results), suggesting that such cues were not used by the rats. Partial reinforcement also slows extinction learning in the unreinforced probe trials, and this method has been previously used to increase the
number of possible probe trials run without inducing extinction (Bateson & Matheson 2007; Matheson et al. 2008). The duration of this phase depended on individual learning. Rats were moved onto the next phase when they completed at least three out of four trials correctly per day for 5 days in a row, at which point we assumed they had learned the discrimination.

In phase F, the same protocol was followed as in phase E except that during the randomly chosen unrewarded trial, an intermediate grade of sandpaper (P180) was used as a probe instead of the sandpaper that normally corresponded to this reward. These probe trials continued for 5 days (total of five probes per rat per week). Owing to individual learning rates, rats entered and finished phase F at different times. During the interim, those that finished first were moved back onto phase D (D2), with the reward always buried at the bottom of the sand.

Phase G began when all rats had completed phase F. At this stage, rats were already housed in two social groups, but in phase G (1 week), these groups were randomly allocated as either ‘enriched’ or ‘unenriched’ treatment groups. Those in the enriched group were moved into a larger, plastic-based metal cage (70 x 45 cm and 54 cm high; ‘the manor’, Pet World Direct, Eye, Suffolk, U.K.) lined with a deep layer of wood shavings and enrichments were added (two cardboard tubes, two cardboard houses, four wooden blocks and one plastic house (57.2 x 18.4 cm and 18.4 cm high; Super Pet, Elk Grove Village, Ill., U.S.A.)). Those in the unenriched group were moved to a new cage that was identical to their old one (61 x 43.5 cm and 21.5 cm high, lined with wood shavings but containing no environmental enrichment). During this phase, rats were trained using the same protocol as phase D (D2), to ensure they continued to retain the task.

After 1 week of treatment (enrichment/no enrichment), rats entered phase F2. This followed the same protocol as phase F, with four trials a day per rat for 5 days, with one probe trial per day.

Data Analysis

Data were analysed using general linear models (GLMs). In all cases, we checked the underlying assumptions of normality of error and homogeneity of variance. Given no evidence to suggest that these assumptions were violated, we proceeded with analysis of untransformed data. Nonsignificant factors were removed in a stepwise manner to produce minimal models. Models are presented in full here; results are for minimal models.

The first GLM assessed the effects of treatment (enriched versus unenriched), time point (pre- versus post-treatment), treatment × time point and rat (a random variable nested within group) on the number of optimistic choices (i.e. responding as if on a chocolate trial during a probe trial) rats made during the probe trials (during phases F and F2); a second GLM assessed the effects of treatment, experimental phase (all phases) and rat (a random variable nested within treatment) on weight; a third looked at the effect of treatment on number of trials to learn the task presented in full here; results are for minimal models.

There was a significant main effect of experimental phase on time to exit the tunnel ($F_{6,1864} = 13.61, P < 0.01$), time to choose a bowl ($F_{6,1838} = 20.94, P < 0.01$) and time to choose the correct bowl ($F_{6,1728} = 27.57, P < 0.01$). Post hoc tests revealed that rats took longer to exit the tunnel in phase C compared with all other phases, and longer to choose a bowl and locate the correct bowl during phases C and D compared with all other phases (Fig. 3a–c). There was a significant effect of reward (chocolate versus Cheerio) on time taken to exit the tunnel ($F_{1,1864} = 14.17, P < 0.01$), time to choose a bowl ($F_{1,1838} = 51.45, P < 0.01$) and time to choose the correct bowl ($F_{1,1728} = 53.76, P < 0.01$), with rats moving faster during the chocolate than the Cheerio trials (Fig. 4).

DISCUSSION

When moved to an enriched environment, rats responded more optimistically to an ambiguous stimulus, meaning that they chose to forage more frequently in a location previously associated with a more highly valued food reward. This increase in optimism was not observed in control rats that continued to experience unenriched conditions, and they preferentially chose to forage in a low-value reward location in response to the same ambiguous stimulus. These findings show for the first time that provision of

Figure 2. Mean number of optimistic choices during five probe trials pretreatment and post-treatment for enriched and unenriched groups. Error bars represent 1 SE. Bars connected by an asterisk are significantly different from one another.
environmental enrichment can induce an optimistic cognitive bias in nonhuman animals.

The above results were obtained using a novel judgement bias task modelled on an analogous approach recently developed for starlings (Matheson et al. 2008; Brilot et al. 2010). The task involves animals learning a conditional discrimination whereby they had to learn the correct response necessary to obtain a food reward given the stimulus present during approach (rough or smooth sandpaper). One stimulus was associated with a higher-valued reward (chocolate) than the other (cereal). Once rats were trained on this discrimination, judgement bias was assessed by exploring the rats’ responses to a novel, ambiguous stimulus (an intermediate grade of sandpaper); a rat was defined as optimistic if it chose the response appropriate to the stimulus associated with the higher-valued reward. This task has a number of advantages over some previous tasks. Unlike previous go/no-go tasks (e.g. Harding et al. 2004; Bateson & Matheson 2007), this task requires the rat to make an active response to the ambiguous stimulus (an intermediate grade of sandpaper); a rat was defined as optimistic if it chose the response appropriate to the stimulus associated with the higher-valued reward. This task has a number of advantages over some previous tasks. Unlike previous go/no-go tasks (e.g. Harding et al. 2004; Bateson & Matheson 2007), this task requires the rat to make an active response to the ambiguous stimulus, thus reducing the difficulties in interpretation of no-go responses (see Matheson et al. 2008 for a discussion). Additionally, the task uses stimuli (textures and odours) likely to be particularly salient to rats, with the intention of reducing the time required by the rats to learn the necessary discrimination (see Brilot et al. 2009 for a similar approach in starlings).

To verify that the rats had learned the conditional discrimination, and were not using any cues directly associated with the rewards themselves (e.g. the odour of the chocolate) to locate them, we...
tested the rats’ performance in extinction (during phase E). Trials were inserted in which rewards were randomly removed, and we found no effect of rewards being present or absent on whether a rat’s first choice was correct or incorrect. We also showed that rats responded faster as training progressed (exiting tunnel, choosing bowl, finding correct bowl), indicating that they were learning the task, and importantly, that there was no evidence of extinction when unreinforced trials were introduced (see Brilot et al. 2010; Doyle et al. 2010). Overall, rats were significantly faster at responding when the reward was a chocolate drop (high reward) compared with a Cheerio (low reward). This difference in latency suggests that we had correctly determined the chocolate drop as the high-value reward, and that it was clearly discriminable from the low reward.

Over the course of this study, the rats were housed in two stable social groups. In the critical treatment phase of the experiment (phase G), one group (enriched) was moved to a new cage furnished with various forms of environmental enrichment, whereas the other group (unenriched) was moved to a new cage identical to their old one. We chose this design because we judged that it would be less disruptive to task acquisition and performance to maintain stable social groups throughout. However, concern has been raised over using the behaviour of individuals from the same group as independent measures (e.g. Weary & Fraser 1998). It has been argued that behaviour of individuals from a particular group may not be independent, owing to factors such as closer similarities in genetics and shared social interactions. Hence, in our study, it could be argued that some aspect of the cagemates’ social interactions, perhaps related to the switch to an enriched environment but not to enrichment per se, was responsible for the observed results. With only one enriched and one unenriched cage, we effectively have a sample size of one for testing the effect of enrichment, and therefore cannot statistically dissociate the specific effects of enrichment from other possible differences present between the two groups of rats.

In response to the above potential criticism of our study, we make the following points. First, care was taken that the two groups of rats did not differ in any consistent way (other than the imposed treatment). Genetically, these animals came from the same stock and were randomly allocated to the two groups. We also showed that there was no difference between the two groups of rats in either rate of task acquisition or weight gain over the course of the experiment. Second, it was not possible for there to have been a direct effect of social interaction during the task, as animals were tested individually. However, rats were with their companions before and after task completion, which could theoretically influence their performance in the task. Any modifications in social behaviour induced solely as a consequence of housing environment can be seen as part of the enrichment. In future studies, it would be interesting to tease apart which aspects of enrichment are the most important in modifying behaviour: for example, social versus physical enrichment. Finally, many previous studies employing proper replication have shown positive welfare benefits of environmental enrichment in laboratory rats (e.g. Fernandez-Teruel et al. 2002; Van Loo et al. 2002; Burman et al. 2006; Fox et al. 2006; Hansen et al. 2007). We were merely using this proven effect to manipulate the state of our rats in the current study. Therefore, the lack of replication of the affective manipulation we used does not devalue our main finding, that provision of environmental enrichment is associated with an increase in optimism.

A previous study used a cognitive bias task to investigate starlings’ responses to being moved from unenriched to enriched cages, but found no evidence for an increase in optimistic responses (Bateson & Matheson 2007). A potentially significant difference between the two studies that could account for the difference in findings is that the starlings had extensive experience of enriched cages and aviaries before the experiment. By contrast, the rats used in the current study were experiencing enriched conditions for the very first time during the treatment phase. It is therefore possible that being moved from unenriched to enriched cages was accompanied by different emotional responses in the two experiments: the rats may have experienced a greater emotional response than the starlings owing to the novelty of the enrichments. Further experiments are needed to test this hypothesis.

Our results provide further support for the welfare benefits afforded by the provision of environmental enrichment for captive animals. Previous studies have shown that enrichment leads to reduction of stereotypies and signs of apparent frustration (Balcombe 2006; Mason et al. 2007). By analogy with data from humans, our results suggest that provision of environmental enrichment might also engender a more positive affective state in rats. They further suggest that lack of enrichment is likely to be associated with poor welfare and negative affect. Before treatment when all rats were housed in unenriched cages, rats from both groups chose to forage predominantly (more than 50% of the time, or chance) in the low-reward location in response to the ambiguous stimulus (the pessimistic response). It might appear that an alternative explanation for this finding is that the ambiguous probe used (intermediate-grade sandpaper) was in fact closer in texture to the low-reward grade of sandpaper. However, this is highly unlikely, as the grade of sandpaper (coarse or fine) associated with the high or low reward was counterbalanced between groups. Our results therefore add to previous work in starlings and rats showing that animals in unenriched housing typically exhibit more pessimistic cognitive biases (Bateson & Matheson 2007; Burman et al. 2008a). This suggests that, potentially, present provision of standard laboratory housing is inadequate for the maintenance of good mental welfare in laboratory animal species studied thus far. Around 3.7 million scientific procedures are started annually on laboratory animals, the majority using rodents (77%, according to Home Office statistics: Office for National Statistics 2008). Owing to the vast numbers of such animals used in scientific research, we have a responsibility to safeguard their mental as well as physical welfare, not just from an ethical standpoint but also to ensure the quality and consistency of experimental data.

In conclusion, we have developed a novel, simple foraging task that can be used to measure cognitive bias in rats. We have shown that rats trained on this task show pessimistic responses when housed in unenriched cages but switch to optimistic responses when moved to enriched cages. Our task therefore has potential for detecting both negative and positive affective states in animals. The further validation and use of this task will be of great importance for the assessment of animal welfare and may also represent an improved method for assessing the preclinical efficacy of potential novel mood-altering therapeutics.

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